THE RELATIONSHIP BETWEEN BODY WEIGHT AND GROWTH HORMONE GENE POLYMORPHISM IN INDIGENOUS TURKEYSOF NIGERIA

Abstract

The candidate gene approach is a valuable tool for improving breeding programs, particularly in poultry, by identifying traits for enhanced production performance. This study investigates the growth hormone (GH) gene as a potential determinantof body weight in Nigerian indigenous turkeys. DNA was extracted from 60 twelve-week-old turkeys using blood samples, and a 466bp DNA fragment was amplified via PCR with primers designed from chicken sequences. The PCR product underwent Msp1 restriction enzyme digestion and was visualized on a 1.5% agarose gel. Genotypes associated with body weight were assessed using a General Linear Model. The GH/Msp1 locus revealed Two alleles (A and G) and three genotypes (AA, AG, and GG), with allele G being predominant. Genotype AG had the highest frequency (0.55), followed by AA (0.42), and GG had the lowest frequency (0.03). However, the least square means analysis showed that the polymorphism in the GH gene of the studied population was not significantly associated with body weight ($p> 0.05$). Nonetheless, the polymorphic nature of the GH gene in Nigerian turkeys suggests potential for further exploration in quantitative traits.

Keywords:Polymorphism, PCR-RFLP; Growth Hormone gene (GH); Nigerian indigenous turkey; bodyweight.

Introduction

Genotyping animals for every genethat encodes polygenic trait seems unreasonabletherefore it is important to focus on genes having effects that is responsible for a significant part of genetic variation observed*(Andrés LegarraandVitezica, 2015).*

Indigenousor native poultry exhibit considerable functional andgenetic significance, owing to their possession of genetic constituents that might have been relinquished within the enhanced gene reservoir. They harbor genetic variations that are either non-existent in contemporary improved breeds or that persist within their scarce progenitors, which sometime hold commercial value*(Sun et al., 2022).*With rising global demand for Turkey's products, its production is both important and profitable in agricultural industry, which makes it crucial in the economy of the country and extensive work is required to improve the economic traits through modern techniques that aid in selection *(Ahmad et al., 2019).*
Growth, carcass quality, meat quality, and reproductive qualities are just a few of the traits that have been linked to genes or markers through advancements in the science of molecular genetics in livestock animals*(Yurnalis et al., 2017).*

The candidate gene approach iseffectivefor genetic advancement in breeding programs, particularly for poultry.Before characterization for genetic improvement have been done using phenotypic approach but since the advent of molecular method such as candidate gene approach, it has made breeding for desirous trait faster and better in the Nigerian indigenous turkey. Work that has been done includes the use of candidate genes such as the myostatin gene *(Fijabi et al., 2020)* and IGF-1*(Oyewole et al., 2018)*. Investigating relationships between gene polymorphisms and economically significant features in farm animals can be done extremely well using this strategy*(Khalil et al., 2021)*. The GH gene has been identified as a candidate gene in numerous research for traits related to growth and carcass in domestic animals (cattle) and poultry(Thai broilers)*(Anh et al., 2015); (Akhmad Dakhlan et al., 2022); (Wang et al., 2023).*

The growth hormone is a protein with apolypeptide chain consists of 191 amino acid residues with a molecular weight of about 22,000. Among the array of candidate genes, the growth hormone gene (GH) appears promising. This gene encodes the growth hormone known as somatotrophic hormone, which has a wide-ranging influence on cellular function throughout the body which includes enhancing protein, DNA, and RNA synthesisand alsofacilitating glycogen biosynthesis*(Singh et al.,2015)*.Therefore, the use of candidate gene approach may increase efficiency in identifying the desired traits needed to improve production performance of Nigerian indigenous turkeys.

The purpose of this study is to detect polymorphism in growth hormone gene of the Nigerian indigenous turkeys using PCR-RFLP technique.

Materials and Method

At random, 60 indigenous Nigerian turkeys were chosen for the experiment. Body weight measurements were taken at 4, 8, and 12 weeks, and whole blood samples were obtained at 10 weeks of age through the jugular vein into EDTA tubes, which were immediately placed into an ice box and transported to the laboratory.
DNA was extracted by usingZymo mini prep kit following manufacturers protocol. The quality and concentration of the extracted DNA was assessed using agarose gel electrophoresis and spectrophotometer respectively.

PCR-RFLP ASSAYS

GH gene was amplified using primers based on the sequence of chicken. The PCR primer5’-ATCCCCAGGCAAACATCCTC(forward)and 5’-CCTCGACATCCAGCTCACAT (reverse)for Msp1 site was used (Thakur *et al* 2006).The PCR for the GH gene was prepared in a total volume of 25 µl. The PCR products with length 466 bp were digested at 37°Cwith Msp1restriction enzyme for 15min and then inactivated at 80°C for 20 minutes. We ran the digested product on electrophoresis using 1.5 % agarose for 30 minutes after which it was viewed under the gel documentation system.

Statistical Analysis

Genotype information was processed in Excel and determined using Pop Gene Version 1.31 software as described by *Yeh et al. (1999)*. Hardy-Weinberg equilibrium test was calculated using chi-square test through Pop Gene Version 1.31 software *(Yeh et al., 1999)*. The effect of GH genotypes on body weight was analyzed using the GLM procedure in SAS ver. 9.1 using Least Squares Means (LSM).

Statistical Model

$Y_{ij}=\mu+gi+\epsilon ij$

Where Y_{ij} = observed trait (body weight)

μ= the overall mean,

G_i= fixed effect of polymorphic variant,

e_{ij}= residual effects associated with jth observation of ith genotype

Results

Table 1: Genotype and allele frequency of GH gene in Nigerian Indigenous Turkey

			FREQUENCY				
		Genotype			Allele		
	No	AA	AG	GG	A	G	X^2(HWE)
Turkey	60	0.42	0.55	0.03	0.6917	0.3083	***

HWE = Hardy-Weinberg Equilibrium, *** = P< 0.05
Note: X^2 = chi-square test, G^2 =likelihood ratio test

Table 2: Least square means and standard error of mean of body weight (g) of Nigerian indigenous turkey.

Age(weeks)	Body weight (g)		
	AA (n=25)	AG (n=33)	GG (n=2)
4	298.60±8.07	311.09±6.60	269.00±40.00
8	567.92±20.40	569.09±16.38	579.50±51.50
12	837.2±36.49	836.1±30.35	885.5±114.50

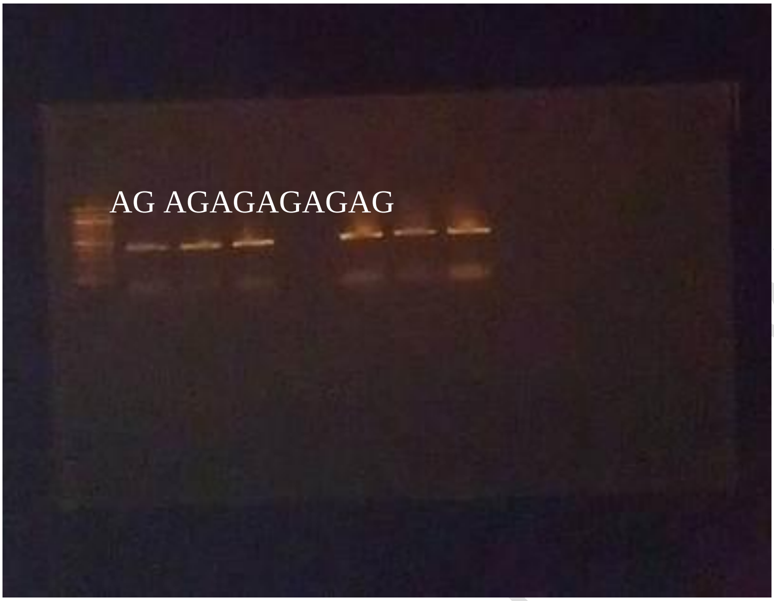

Figure 1: Electrophoresis patterns of intron 1 of the growth hormone (GH) gene of Nigerian indigenous turkey obtained by digestion using Msp1restriction enzyme.

Discussion

Enhancing economic traits in poultry hasbecome very important because of its relatively short life cycle and large number of progeny*(Nassar*, 2018*)*. Identifying and utilizing molecular markers provides lots of potential for genetic improvement in selection programme. In local or domestic breeds of animals, genetic diversity allows breeders and researchers to maintain genetic diversity while improving production by developing new traits in response to environmental changes like heat stress and diseases*(Olschewsky et al., 2021)*. Recently the DNA polymorphism of different genes with its effect in chickens and other animals has been studied. In chicken GH gene, several polymorphisms have been discovered at the intron which includes intron1, 3 and 4 *(Kulibaba et al., 2017);(Wheto et al., 2023)*.

In this study, as shown in table 1, genotype GG was found to have the lowest frequency among the population while AG has the highest frequency, and this agrees with the report of *Wu et al. (2012)* in Cherry Valley duck whereit was observed that the TT genotype has the lowest genotypic frequency (0.30) while CT has the highest genotype frequency but contrary to *Nie et al.(2005)* whose reports says that GG genotype has the highest genotype frequency and AA has the lowestgenotype frequency in Leghorn and Taihe silkies chickens. The difference observed may be due to the population structure that was sampled which is different.

The probability of random mating was estimated by chi-square (x^2) in the Nigerian indigenous population test to examine Hardy Weinberg equilibrium (HWE) which was found to be significant ($p < 0.05$), thisshows that the population deviates from Hardy Weinberg equilibrium, which islike the findings of *Wu et al. (2012)* on Cherry Valley duck and Muscovy duck populations. There are several reasons that could be responsible for this deviation which could be the fact that the sampled population had experienced intensive selection, assortative mating, migration within the populationand mutation. Deviations of genotype frequencies from the Hardy–Weinberg equilibrium isexpected for loci with impact on traits in population under selection *(Abramovs et al., 2020);(Fijabi et al., 2020).*

For this research, the GH gene was investigated as a candidate gene to evaluate the polymorphisms and its association with body weight in Nigeria indigenous turkey populations. Two alleles was discovered with three genotypes which are A and G allele, and AA, AG and GG genotypes, the same result was reported by Wu *et al.*, (2012)andAnh *et al.*, (2015) in Thai broiler though contrary to Thakur(2006) who discovered three alleles and six genotypes in domestic poultry strains.Thiscan be due to environmental effects which may have contributed to selection for this genotypes in different population. In table 2, we assessed the association between different genotypes with weight at 4, 8 and 12 weeks. The results showed that GG was associated with superior non-significant ($p > 0.05$) body weight with slight numerical increase over genotype AA and AG at 8 and 12 weeks.This is supported byWu *et al.* (2012) report on avian growth hormone gene polymorphism effect on growth and carcass characteristics in duck, it was shown that TT genotype has higher body weight than CC and TC genotype at 56days.

In Pitalah Ducks, the dominant allele is G, and the most frequent genotype is AG which is similar to this study on Nigerian indigenous turkey. Three genotypes (AA, AG, and GG) were discovered as shown by Yurnalis *et al.* (2017).It was observed that AG genotype was characterized by higher body weight than the GG genotype which is contradictory to the findings of this research. No significant difference was found in this study between the body weight and the genotypes which is contrary to the findings ofSinpru *et al.* (2021), where a significant difference between body weight and genotype was reported.Polymorphisms of GH gene have been reported in chickens at intron1, 3 and 4. In thepresent study, polymorphism was discovered at msp1 site in the Nigerian indigenous turkey and this is in accordance to Ghelghachi *et al.* (2013) that discoveredpolymorphisms in intron1 regions of the Arain chicken GHwhere he discovered three alleles (A_1, A_2 and A_3) and six genotypes.Ghelghachi *et al.* (2013) reporteda significant difference between Growth hormone gene andbody weight at 6 weeks.InAwassi Lambs where the genotype of GH gene did not have a significant effect on weaning weight, body measurements, or the growth rate of lambs*Qasimi et al.* (2019).However,further research should be conducted on Nigerian indigenous turkey using markers associated withquantitative traits to elaborate more on therelationship of GH gene polymorphism with body weight.

Conclusion

In summary, it can be concluded that in Nigerian indigenous turkey population, the growth hormone gene exhibit a moderate to high level of polymorphism. This indicates the potential for genetic enhancement through selective breeding initiatives. The result from this preliminary study can serve as a foundational resource for further investigations and inform conservation strategies aimed at preserving the genetic diversity of Nigerian indigenous turkey populations.

References

AbramovsN,BrassA,Mayada Tassabehji. Hardy-Weinberg Equilibrium in the Large Scale Genomic Sequencing Era. *Frontiers in Genetics, 11*. https://doi.org/10.3389/fgene.2020.00210

Ahmad S, MahmudA, Hussain J, Javed, K. Productive Performance, Egg Characteristics and Hatching Traits of Three Chicken Genotypes under Free-Range, Semi-Intensive, and Intensive Housing Systems. *Brazilian Journal of Poultry Science*, 2019,*21*(2). https://doi.org/10.1590/1806-9061-2018-0935

Akhmad Dakhlan, Kusuma Adhianto, Sulastri Sulastri, Kurniawati D., Ratna Ermawati, Tri Doni Saput. Mapping Growth Hormone Gene of Body Weight Krui Cattle in Pesisir Barat Regency Lampung, Indonesia. *Pakistan Journal of Biological Sciences*, 2022:*25*(8), 741–747. https://doi.org/10.3923/pjbs.2022.741.747

Andrés Legarrab, Vitezica, Z. Genetic evaluation with major genes and polygenic inheritance when some animals are not genotyped using gene content multiple-trait BLUP. *Genetics Selection Evolution*, 2015:*47*(1). https://doi.org/10.1186/s12711-015-0165-x

AnhNTL, Kunhareang S, Duangjinda M. Association of chicken growth hormones and insulin-like growth factor gene polymorphism with growth performance and carcass traits in Thai broilers. *Asian-AustralasianJournal of Animal Sciences*, 2015;28 (12): 1686-1695.

FijabiOluwatobi,Osaiyuwu Osamede, Mabel Akinyemi,Shobanke Ibilola,Khadijat Oyewola,NwokorieG,EwuolaKM. The Agricultural Science Society of Thailand Myostatin gene polymorphism and its association with body weight in Nigerian indigenous turkey. *Thai Journal of Agricultural Science*. 2020):53:58-66.

GhelghachiAA, SeyedabadiHR, Lak A. Association of growth hormone gene polymorphism with growth and fatness traits in Arian broilers. *International Journal of Bioscience*, 3: 216-220

ISAAC U. C. (2021). Phenotypic, genetic and environmental correlations between body weight and linear body traits of chicken genotypes. *Agrobiological Records*, 2013;*4*:32–43. https://doi.org/10.47278/journal.abr/2020.026

Khadijat Oyewole,MabelAkinyemi,Osaiyuwu Osamede, Fijabi Oluwatobi,Nwokorie GI, EwuolaKM. Association between igf- 1 gene polymorphisms and body weight in Nigeria locally adapted turkeys.*Nigerian Journal of Genetics*. 2018);32(1):207-211.

KhalilMH, IraqiMM,Saleh MS. Molecular Approaches of Candidate Genes in Genetic Improvement Programs in Poultry. *Ḥawliyyaāt Al-’Ulūm Al-Zirā’iyyaẗ Bi-Muštuhur*, (2021): *59*(2), 9–30. https://doi.org/10.21608/assjm.2021.183334

KulibabaPO, Liashenko Y &YurkoPS.Novel AluI-polymorphism in the fourth intron of chicken growth hormone gene. *Cytology and Genetics*, 2017:.*51*, 54–59. https://doi.org/10.3103/s0095452717010091

Nassar FS. Poultry as an Experimental Animal Model in Medical Research and Pharmaceutical Industry. *Biomedical Journal of Scientific and Technical Research*, 2018.*2*(3). https://doi.org/10.26717/bjstr.2018.02.000751

OlschewskyA,Hinrichs D. An Overview of the Use of Genotyping Techniques for Assessing Genetic Diversity in Local Farm Animal Breeds. (2021). Animals. 11(7):2016. doi: 10.3390/ani11072016. PMID: 34359144; PMCID: PMC8300386.

Qasimi A, Amad Falah Hassan, Khudair BY. Effect of IGF-1 and GH Genes Polymorphism on Weights and Body Measurements of Awassi Lambs in Different Ages. *Mağallaẗ Al-Baṣraẗ Al-ʿulūm Al-Zirāʿiyyaẗ*, 2019.*32*(1): 39–46. https://doi.org/10.37077/25200860.2019.125

Singh PP, Tomar SS, Thakur MS, Kumar, A. Polymorphism and association of growth hormone gene with growth traits in Sirohi and Barbari breeds of goat.*Vet World*, 2015;.8:382-387. http://doi.org/10.14202/vetworld.2015.82-387

Sinpru P, BunnomR, Poompramun C, Kaewsatuan P, Sornsan S, Kubota S, Molee W, Molee A. Association of growth hormone and insulin-like growth factor I genotype with body weight, dominance of body weight, and mRNA expression in Korat slow-growing chickens. *Animal Bioscience*, 2021; *34*(12). https://doi.org/10.5713/ab.20.0729

Sun Y, Li Y, Zong Y, Gamal MK Mehaisen, Chen J. Poultry genetic heritage cryopreservation and reconstruction: advancement and future challenges. *Journal of Animal Science and Biotechnology*, 2022;13(1). https://doi.org/10.1186/s40104-022-00768-2

Thakur MS, Parmar SN, Toenkhomba TC, Strvastava PN, Joshi CG. Growth hormone gene polymorphism in Kadaknath breed of poultry. *Indian Journal of Biology*, 2006;5:189-194.

Wang F, Wu X, Ma X, Bao Q, Zheng Q, Chen M, Guo X, Liang C, &Yan P. The Novel Structural Variation in the GHR Gene Is Associated with Growth Traits in Yaks (Bos Grunniens). *Animals*, 2023;*13*(5):851–851. https://doi.org/10.3390/ani13050851

Wheto Mathew, Ayodele Oguntuase, Sunday Adenaike, CHIMANG.,Henry Ojoawo, Yakubu Abdulmojeed, Adebambo Ayotunde Olutumininu, Adebambo Olufunmilayo Ayoka. Sequence analysis of exon 1 and intron 1 of growth hormone gene in six chicken genotypes raised in tropical environment. *Biotechnology in Animal Husbandry*, 2023: *38*(1):41–54. https://doiserbia.nb.rs/Article.aspx?ID=1450-91562201041W

Yeh FC,Young R. Popgene version 1.31: Microsoft-based Freeware for population Genetic Analysis. University of Alberta, Edmonton, Canada.1999.

Yurnalis H,Sabrina. Polymorphisms of Growth Hormone Gene Exon 1 and their associations with Body Weight in Pitalah and Kumbang Janti Ducks. *International Journal of Poultry Science*, 2017;16:203-208.